

THE METABOLISM OF RADIONUCLIDES BY MARINE ORGANISMS.  
I. THE UPTAKE, ACCUMULATION, AND LOSS OF  
STRONTIUM <sup>89</sup> BY FISHES

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The revolution in biology which occurred about twenty years ago as a result of the utilization of isotopes has led to some problems which could hardly have been foreseen at the time. Biologists can no longer use radioactive material simply as a tool with which to solve specific problems; they are now forced to consider the effects of radioactivity which is introduced beyond their own control into an environment which they are studying. This becomes particularly important to marine and fresh water biologists who are interested in physiological or ecological problems which may be influenced in certain regions by an increase in radioactivity above the background which existed before the first atomic detonation at Alamogordo in 1945.

The weapons testing program of several nations, regardless of the type of blast, has increased the radioactivity of the seas. Underwater detonations, of course, contribute the largest percentage of their radioactivity directly to the water, but most of the fall-out from aerial bursts can be expected to appear ultimately in the ocean, since the land area of the earth is only about 30 per cent of the total. Run-off from the land will also increase the radioactivity of the seas. Far more important than the radioactivity which appears as a result of weapons testing, however, is the radioactivity which inevitably will be introduced into the oceans from nuclear power plant wastes and atomic-powered ships.

There are several important reasons for studying the metabolism of fission products and other radionuclides in marine organisms. First, several fission products are known to be potential hazards from a public health standpoint (N.B.S. Handbook 52). Second, almost nothing is known about the metabolism of these radioelements by marine species. Third, we cannot tell at present what potential ecological effects may be brought about through the deleterious action of radiation on the marine biota, but the possibility exists that some adverse changes, such as those which apparently occurred in White Oak Lake (Krumholz, 1956), might occur in estuaries and other inshore regions. It is therefore important to study these problems now, before the oceans become polluted with radioactivity, because the changes which may occur will be irreversible at least for several centuries.

The problems raised by the above considerations can best be solved by studying the metabolism of these radionuclides not only in individual organisms, but also in relation to the various trophic levels by way of the food chains. As desirable as such studies may be, it is impossible to undertake investigations of this magnitude

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for the entire marine biota. Thus, because of our special facilities at the Hawaii Marine Laboratory, we have confined our research to fishes which are representative of three distinct marine habitats, and include herbivores belonging to the second trophic level, and carnivores from the third and fourth trophic levels.

Strontium was selected for our initial studies for two reasons. First, it is chemically similar to calcium, and is therefore a "bone seeker." As such, if radioactive, it may interfere with the blood cell formation of many animals. Second,  $\text{Sr}^{90}$  has a half-life of about 28 years, so that the deposition of an atom of  $\text{Sr}^{90}$  into a tissue which has a slow rate of turnover may result in radiation exposure for the entire life of the animal. These characteristics make radiostrontium a particularly hazardous fission product. In these experiments we have used  $\text{Sr}^{89}$  because of its much shorter half-life ( $\sim 53$  days) which decreases the danger of contamination in the laboratory.

The particular objective of the present study was to measure the uptake, accumulation, and loss of radiostrontium by the various tissues and organs of selected species of fish when the isotope was given orally, by intramuscular injection, and by the immersion of the fish in sea water enriched with  $\text{Sr}^{89}$ .

#### MATERIALS AND METHODS

The large pelagic fishes used in these experiments consisted of the black skipjack (*Euthynnus yaito*), the yellowfin tuna (*Neothunnus macropterus*), and the so-called dolphin (*Coryphaena hippurus*). These species are fast-swimming, wide-ranging carnivores which occupy the fourth trophic level. Among the small fish used, the papio (*Carangoides ajax*), and the aholehole (*Kuhlia sandvicensis*), are small carnivores common along the reefs and shores in the Hawaiian Islands and occupy the third trophic level primarily. The aholehole is also able to adapt itself to brackish water environments, and has even been found well into fresh water streams (Tester and Takata, 1953; Tester and Trefz, 1954). The third small species used, *Tilapia mossambica*, is a sluggish fish, predominantly herbivorous, but facultatively omnivorous, and may be placed in the second trophic level. It prefers brackish water, but is well adapted to either fresh water or sea water.

Carrier-free strontium<sup>89</sup> was obtained from Oak Ridge and fed to the large fishes by filling gelatine capsules with cracker crumbs and a measured quantity of the isotope solution. The capsule was sewn into a small piece of fish muscle which was held just under the surface of the water by a weak thread. As the fish swallowed the bait, the thread was broken off. In this way the capsule could be given to a particular fish. In some instances small fishes were force-fed a gelatine capsule prepared in the same way. Others were fed by incorporating a measured amount of  $\text{Sr}^{89}$  into a gelatine solution which was allowed to solidify in a small plastic tube. The tube was put into the fish's stomach and the gelatine was extruded with the aid of a syringe. A dose solution was prepared by extruding the same quantity of radioactive gelatine into a volumetric flask.

The large fish were perfused with a mixture of two parts sea water and three parts distilled water. The brain, eyes, spinal cord and integument were removed, and the excised internal organs were further soaked in distilled water until no blood was apparent in the water. All the rinsings were added to the blood. The gut was opened, and any material remaining in it was flushed out. Only the eyes and

the visceral organs were removed from the small fish which were not perfused.

The remainder of the fish, consisting of muscle and skeleton for the large fish, or muscle, skeleton and integument for the small fish, was then put in a pressure cooker, brought to 20 pounds pressure and allowed to cool. After this treatment, the muscle was easily removed from the bones, and any flesh remaining on the gill arches was removed with warm formamide. Control experiments indicated that no leaching or loss of strontium occurred as a result of the pressure cooker treatment.

Wet weight of organs was obtained without blotting, and the tissues were dried at about 110° C. for 48 hours. The dried tissues were put in a muffle furnace which was brought to about 550° C. The furnace was then shut off and the samples left overnight. A slow stream of air was introduced in the oven to aid combustion.

The ash was ground and spread evenly on aluminum planchettes with the aid of water and a detergent, and dried under infra-red lamps. The samples were

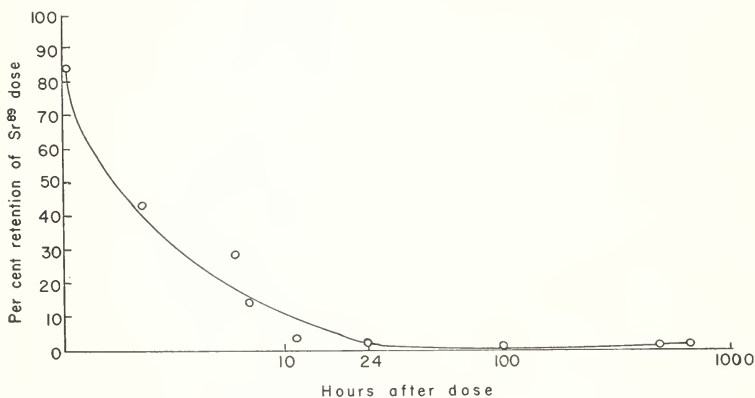


FIGURE 1. The decrease of ingested Sr<sup>90</sup> in pelagic fishes as a function of time.

counted in triplicate when possible, using commercial counters and scalars. A minimum of 2560 counts was taken on each sample, including background. No corrections were made for back-scatter, self-scatter, or self-absorption. The latter is very small at the ash densities which were used (< 5 mg./cm.<sup>2</sup>). The scalars were calibrated daily with Bureau of Standards nuclides, and the only correction applied was for radioactive decay. The counts/minute of the samples were compared with aliquots of the actual dose given in each instance. Specific details for each experiment will be described at the appropriate place.

## RESULTS AND DISCUSSION

### A. Ingestion of Sr<sup>90</sup> by large pelagic fishes

Figure 1 shows that the excretion of a single dose is very rapid: about 50 per cent disappeared within a few hours, and only 1-2 per cent was left after 24 hours. This latter value persisted for the remainder of the experiment which lasted 27 days.

Table I shows the distribution of the  $\text{Sr}^{89}$  in the various organs and tissues of these fishes, and Figure 2 is a graph showing some of these data. This graph is presented as the percentage of radioactivity of the different organs and tissues in terms of the total radioactivity found in the entire fish when it was killed. It is

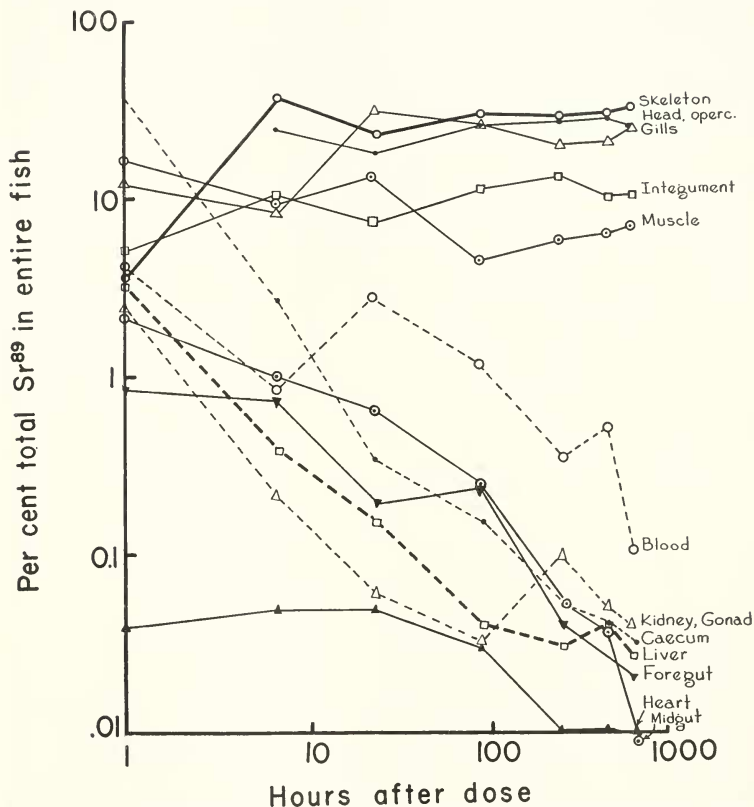


FIGURE 2. The distribution of a single ingestion of  $\text{Sr}^{89}$  in organs and tissues of pelagic fishes as a function of time.

apparent that the tissues are segregated into two groups with regard to strontium retention: the visceral, and the structural. The visceral organs and tissues, including the blood, kidney, foregut, midgut, hindgut, spleen, liver, caecum and heart, show a continuing decrease in radioactivity beginning one hour after the administration of the dose. The structural tissues, including the skeleton, head and

opercular bones, gill arches, integument and muscle appear to concentrate strontium rapidly to a level which is maintained more or less constant for a relatively long period of time. The turnover or excretion of strontium in these structures is therefore slow.

TABLE I

*Accumulation of ingested Sr<sup>90</sup> in the various organs and tissues of pelagic carnivorous fishes expressed as percentage of total activity*

Tissue	Species	EY <sup>1</sup>	EY	EY	CH <sup>2</sup>	EY	EY	EY	NM <sup>3</sup>	EY	EY
	Dose in $\mu$ c	5.55	480	240	51.0	240	464	464	464	371	371
	Duration in hours	1	2½	6	7	11½	24	96	264	480	648
Heart		0.04	0.01	0.03	0.049	0.11	0.05	0.028	0.01	0.014	0.007
Gall bladder		0.05	0.04	0.07	0.10	0.08	0.03	0.0001	0.01	0.004	0.002
Blood		4.21	6.68	15.00	0.85	8.07	2.73	1.14	0.35	0.51	0.12
Gill flesh			0.18	0.91		5.06			2.42	1.42	2.21
Gill bone	12.44		1.34	6.47	8.56	26.39	30.61	25.72	16.80	19.48	22.76
Caecum		37.01	7.67	7.84	2.70	2.64	0.34	0.15	0.05	0.04	0.029
Foregut		0.89	9.32	1.12	0.74	1.03	0.20	0.24	0.04	0.04	0.018
Midgut		2.28	14.16	16.50	1.08	1.48	0.65	0.25	0.05	0.036	0.003
Hindgut		11.78	3.98	21.26	2.26	0.11	0.15	0.024	0.03	0.015	0.016
Gut contents		—	48.32	12.73	0.056	19.65	—	0.10	0.013	—	0.0008
Head, operculum		—	0.41	1.09	24.99	6.28	18.33	24.58	28.18	29.91	24.58
Appendicular skeleton		3.60	0.40	1.19	36.21	8.45	23.69	30.32	29.15	30.47	31.43
Liver		3.34	1.48	3.04	0.39	2.46	0.15	0.04	0.03	0.04	0.027
Spleen		0.20	0.32	1.39	0.08	0.60	0.03	0.008	0.03	0.010	0.003
Tail		—	0.42	0.15	—	0.00	—	—	—	—	—
Brain, spinal cord			0.00	0.01		0.05			1.33	0.030	0.004
Eyes	0.23		0.04	0.06	1.24	0.60	1.66	1.70	2.02	1.34	1.34
Integument		5.28	1.69	0.86	10.20	5.89	7.69	11.37	13.73	10.25	10.51
Integument flesh (aliquot)		—	0.01	0.01	—	0.05	—	—	—	—	0.065
Integument scales (aliquot)		—	0.02	0.02	—	0.11	—	—	—	—	0.091
Gonad			0.09	0.47		0.08	—	0.004	0.03	0.023	0.020
Kidney	2.40		0.08	0.16	0.22	0.09	0.06	0.027	0.07	0.035	0.022
Light muscle			3.23	8.74	4.19	10.01	12.84	3.94	5.26	5.69	5.79
Dark muscle	16.23		0.10	0.86	5.25	0.70	0.72	0.48	0.47	0.63	0.95

<sup>1</sup> EY = *Euthynnus yaito*.

<sup>2</sup> CH = *Coryphaena hippurus*.

<sup>3</sup> NM = *Neothunnus macropterus*.

We do not interpret the departures from a smooth curve for any one organ to indicate a sequential pattern. In other words, a rise in the radioactivity of one organ and the fall of radioactivity in another are not necessarily linked by way of precursor relations. Each point on the graph represents a single fish, and individual differences can most likely account for the small deviations of the curves.

In order to study the sequential pattern of strontium metabolism, a much larger group of fish would have to be used, particularly since it is known that there is a very large difference in the time a food bolus remains in a fish as compared with another fish of the same species living in the same tank.

The rank order of radioactivity in the organ systems of these fishes is: skeleton, gills, integument, muscle and viscera. It is interesting to note that the dark muscle, which has a better supply of blood, has less radioactivity/gram ash than has the light muscle. Similarly, the "specific activity" of the gills, that is, the counts/minute/mg. ash, was considerably higher than that of the axial skeleton. Goldberg (personal communication) has analyzed yellowfin tuna for various metals, and found that the gill arches and filaments had considerably more strontium in them than had

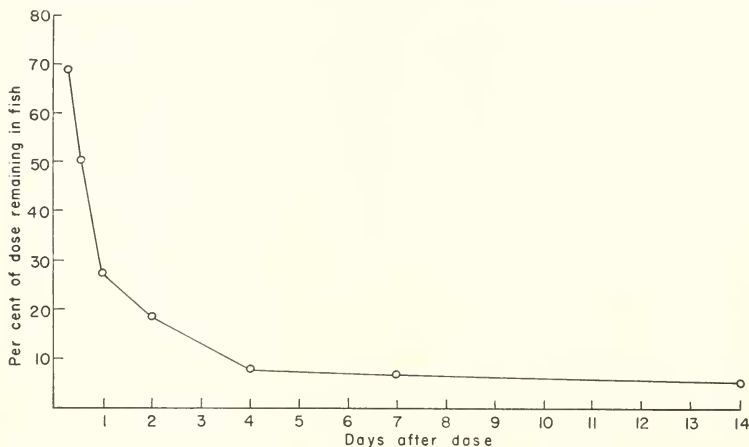


FIGURE 3. The decrease of ingested  $\text{Sr}^{90}$  in *Tilapia mossambica* as a function of time.

the bones. His values for strontium in the gills would therefore be a minimum value, since any flesh adhering to the sample would tend to dilute the strontium concentration. The main chemical difference between the gill arches and the remaining skeletal tissue is the presence of cartilage in the gill rakers. In our own work, and in the work of others (Jones and Copp, 1951), there is a suggestion that cartilage may have a greater capacity to exchange ionic calcium for strontium than has bone. For example, we have found a higher "specific activity" in the eye, which has cartilaginous ossicles, than we have found in skeletal bone. Moreover, Jones and Copp found that the uptake of strontium by the skeleton is more rapid in young rats than it is in adults. It is possible that the explanation of these differences might lie with an increased amount of  $\text{Sr}^{++}$  binding by the protein of the cartilage as compared to that bound by the protein of calcified bones. Perhaps differences in the amount of blood supplied to ossified and cartilaginous tissue, or some other properties of cartilage may also be involved.

TABLE II

Percentage of total radioactivity recovered in various organs of *Tilapia mossambica*.  
(Each horizontal column represents an individual fish given a dose of 20  $\mu\text{c}$ )

Duration	Tissue					
	Integument	Eyes	Visceral organs	Gills	Muscle	Skeleton
2 hr.	10.58	0.26	31.88	28.02	9.44	19.81
	1.73	0.08	88.01	1.43	0.83	7.92
4 hr.	12.54	0.36	28.64	23.50	6.45	28.52
	0.32	0.07	65.81	11.34	10.88	11.58
8 hr.	1.70	0.29	24.41	36.18	6.09	31.34
	10.64	1.38	44.50	6.90	3.72	32.85
12 hr.	8.01	0.24	44.40	15.83	5.46	26.06
	19.66	1.62	11.99	17.10	1.74	47.88
24 hr.	23.07	0.24	42.34	3.65	7.61	23.08
	4.63	1.26	78.36	1.95	1.61	12.19
48 hr.	7.86	0.67	40.92	8.94	4.26	37.36
	25.67	0.32	1.45	9.43	8.96	54.18
7 days	24.93	0.36	1.60	8.60	3.52	60.99
	28.31	0.53	3.57	8.74	4.82	54.03
14 days	22.67	0.31	0.62	4.81	1.62	69.97
	5.79	0.83	3.32	14.35	7.39	68.32
4 days	26.48	0.29	1.76	8.40	2.30	60.77
	23.10	0.16	0.40	5.99	2.17	68.17
7 days	26.71	0.27	0.64	8.08	2.38	61.92
	23.63	0.17	0.93	8.20	3.35	63.72
14 days	21.88	0.22	0.97	8.02	2.26	66.65
	20.84	0.21	0.57	8.28	1.92	68.18
4 days	31.42	0.24	0.17	8.92	1.97	56.73
	29.63	0.41	1.10	10.06	2.10	56.69
7 days	20.39	0.22	0.51	8.07	1.24	69.57

#### B. Ingestion of $\text{Sr}^{90}$ by *Tilapia*

Figure 3 shows that the rate of excretion of  $\text{Sr}^{90}$  by *Tilapia* is much slower than that by the pelagic fishes. About 50 per cent is still present after one day, and the time required to reach a more or less constant level is about four days. The amount

which persists is also larger than that observed with the pelagic fishes, although the variability among the *Tilapia* was fairly large. The *Tilapia* used in these experiments were fed the  $\text{Sr}^{89}$  in gelatine capsules containing cracker crumbs. Occasionally crumbs were observed in the carboys used to hold three of the experimental fish, and therefore the true dose could not be ascertained. The incorporation of the isotope in gelatine for the later experiments has apparently obviated this difficulty.

Table II presents the data concerning the percentage of the total radioactivity recovered in the various organs and tissues. Figure 4 is a graph of this information except that the ordinate is given in microcuries/gram fresh weight of fish.

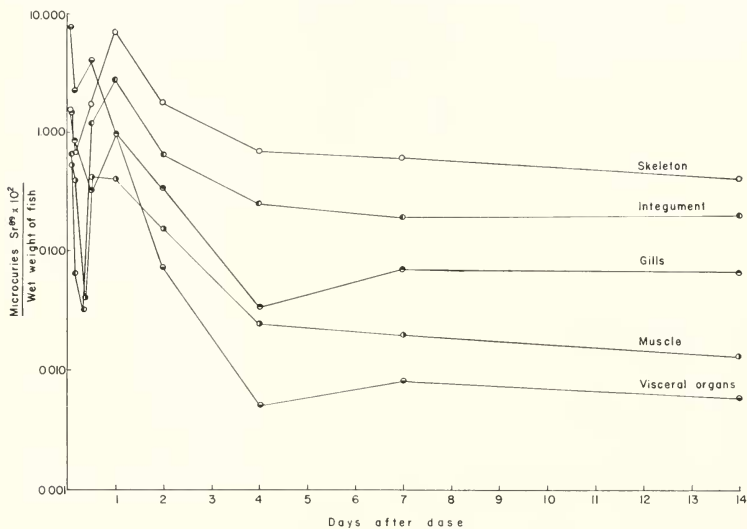


FIGURE 4. The distribution of a single ingestion of  $\text{Sr}^{89}$  in organs and tissues of *Tilapia mossambica* as a function of time.

The structural tissues account for the bulk of the radioactivity where approximately 90–95 per cent of the activity is present in both *Tilapia* and the pelagic fishes. Roughly, about 60 per cent is accounted for by the skeleton, 30 per cent by the integument, 10 per cent by the gills, 2 per cent by the muscle, and 1 per cent by the viscera. A larger percentage of radioactivity is found in the integument of *Tilapia* than in the integument of the other fishes, probably because a larger percentage of the body weight of this species is due to the large scales. The percentage of the total radioactivity in *Tilapia* was found to decrease in the following order: skeleton, integument, gills, muscle and viscera. The order in the pelagic fishes studied was skeleton, gills, integument, muscle and viscera.



### C. The ingestion of $Sr^{89}$ by aholehole

Five aholehole were each fed  $48 \mu\text{c}$  of  $Sr^{89}$  in gelatine capsules and kept in running sea water 24 hours before killing. The entire fish was then dried and ashed, and Table III shows that the results are much more reproducible than they were using *Tilapia*. After 24 hours the latter fish retained approximately the same percentage of the dose as did the aholehole, but the range was between 2 and 20. No further experiments were conducted with aholehole at this time, but because of their apparent superiority as a laboratory animal we plan to use them in experiments which will be reported at a later date.

Although we have completed some experiments on the repetitive feeding of  $Sr^{89}$ , the uncertainty of the exact dose in some instances, and the excessive range of retention by *Tilapia* during short periods have caused us to omit these data here. The results of such experiments on other fish will be reported at a later date.

TABLE III  
Percentage of  $Sr^{89}$  retained by aholehole 24 hours after ingestion

Fish	Per cent of dose
1	10.43
2	7.42
3	9.16
4	5.70
5	7.97
Aver.	8.13

### D. The uptake and accumulation of $Sr^{89}$ injected intramuscularly into tuna and *Tilapia*

Because our results showed that the biological half-life<sup>3</sup> of strontium in fish muscle fell in the same range as that found for the bones and integument, rather than with the soft tissues as was expected, additional experiments were devised to study the retention of Sr by muscle. These experiments also enabled us to study the metabolism of radiostrontium which was introduced by a method other than feeding.

A yellowfin tuna was injected with  $288 \mu\text{c}$  of  $Sr^{89}$  at the base of the pectoral fin. The fish was killed and analyzed after it had been in running sea water for 19 hours. A comparison of the percentage of  $Sr^{89}$  recovered in the organs and tissues of a tuna which received the isotope by injection with that of a tuna receiving an oral dose (Table IV) shows that the digestive organs of the latter were relatively more radioactive than were the corresponding organs of the injected fish. This result is perhaps to be expected, but there are several other outstanding differences in the distribution of the isotope within this time period. The muscle tissue of the fish receiving the injection had almost three times the percentage of  $Sr^{89}$  as had the muscle of the fish receiving the radioisotope orally. The gills of the former, on the other hand, had only about half the percentage of  $Sr^{89}$  as had the orally dosed

<sup>3</sup> We define the term "biological half-life" in this paper to mean the time required for half the labelled strontium to be removed from the tissue or animal in question, exclusive of radioactive decay.

fish. The percentage of radioactivity in the integument and the skeleton of both fish was about the same, and it is not possible to state whether or not the differences found in the remaining organs are significant.

The fact that such a large percentage of the  $\text{Sr}^{89}$  was retained by the muscle suggested that it might be informative to study this process over a longer period of time. Thus, *Tilapia* were injected intramuscularly between the caudal and anal fin with  $\text{Sr}^{89} \text{Cl}_2$  neutralized to about pH 6. The fish were kept in aerated, but not circulating, sea water for the first 24 hours, during which time samples of the water in the aquaria were removed. After 24 hours the fish were put in running sea water and removed at intervals. Figure 5 shows that the radiostrontium was rapidly excreted for about the first twelve hours, and that the rate decreased thereafter.

TABLE IV

*A comparison of the percentage of  $\text{Sr}^{89}$  recovered in the organs and tissues of tuna related to the route of administration*

Tissue	Oral <sup>1</sup>	Injected <sup>2</sup>
Integument	7.69	7.15
Gill bones	30.61	15.25
Gill flesh		1.81
Head, operculum	18.33	14.41
Appendicular skeleton	23.69	23.25
Light muscle	12.84	33.24
Dark muscle	0.72	1.51
Foregut	0.20	0.11
Midgut	0.65	0.36
Hindgut	0.15	0.07
Kidney/gonad	0.06	0.20
Heart	0.05	0.04
Caecum	0.34	0.16
Liver	0.15	0.13
Spleen	0.03	0.02
Gall bladder	0.03	0.37
Blood	2.73	1.79

<sup>1</sup> Skipjack, duration 24 hours.

<sup>2</sup> Yellowfin, duration 19 hours.

An analysis of the percentage of the dose retained by the fish confirmed the fact that after 24 hours comparatively little strontium was excreted, and that most of the strontium remaining was held by the fish for the duration of the experiment. These results are shown graphically in Figure 6. The points on the graph represent the average of three fish. The greater retention of the five-day fish as compared to the one-day fish can be ascribed to individual variation. The range of retention between the one- and the 21-day fish is of the same order of magnitude as the range of retention at any single time interval. In other words, the curve, neglecting individual differences, is very likely parallel to the abscissa.

The internal distribution of the injected  $\text{Sr}^{89}$  requires longer to reach a "levelling-off" than does  $\text{Sr}^{89}$  given orally (Fig. 7). In the former instance, the time required is about one week, whereas in the latter instance the "levelling" occurs within two days. In both situations, however, the percentage of the total radioactivity retained by each of the organ systems is the same.

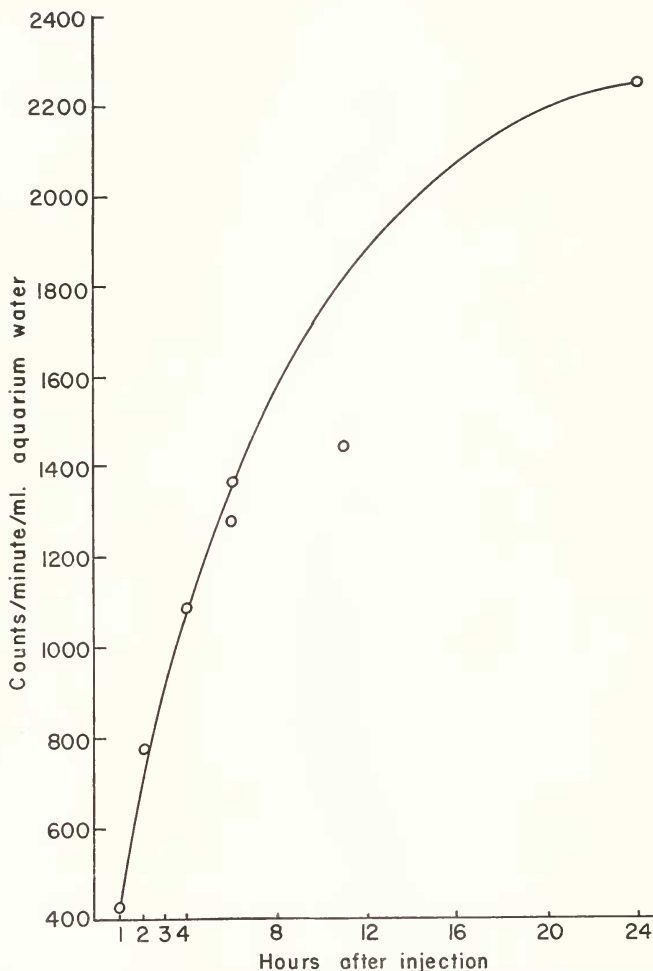


FIGURE 5. The rate of excretion of  $Sr^{90}$  injected intramuscularly into *Tilapia mossambica*.

The aquaria used in these experiments were inverted five-gallon carboys with the bottoms removed. Feces and other solid material thus settled to the neck of the carboy and could be removed through glass tubing which just penetrated the rubber stopper. In this way the feces were removed from the tanks six times during the first 24 hours at each sampling of the tank water. The average total

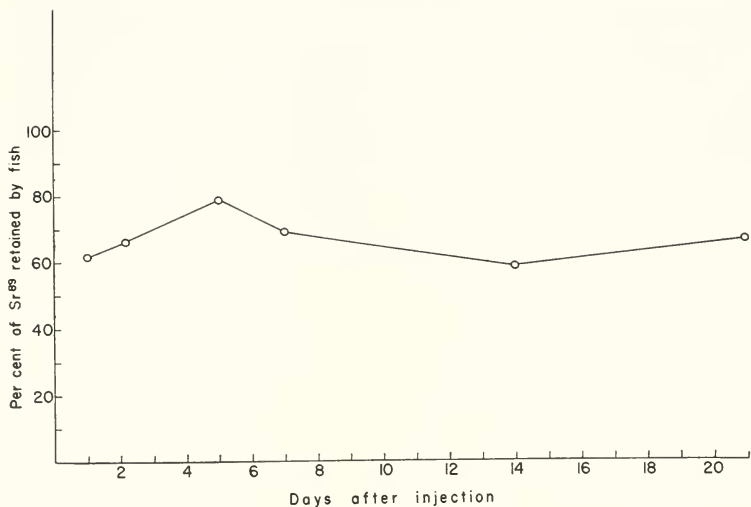


FIGURE 6. The continuing retention of Sr<sup>89</sup> injected intramuscularly into *Tilapia mossambica*.

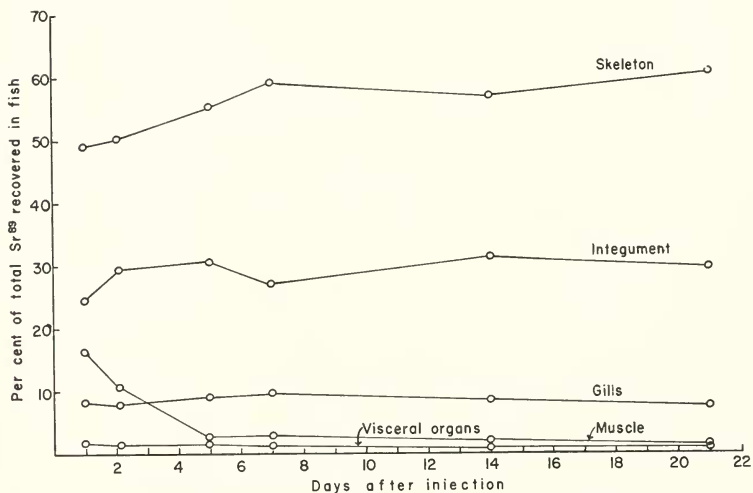


FIGURE 7. The internal distribution of Sr<sup>89</sup> injected intramuscularly into *Tilapia mossambica*.

$\text{Sr}^{89}$  recovered in the feces was 0.35 per cent of the dose at 24 hours, but the amount of leaching is unfortunately unknown.

The injected fish retained a much greater percentage of the dose than did the fish which received the strontium orally. These results suggest that the injected  $\text{Sr}^{89}$  was actually absorbed to a greater extent, and that the absorption of strontium through the gut is not efficient. Slow, continuous diffusion from the site of injection may also allow a larger amount to become incorporated into the various tissues. If the rate of blood supply to the muscles is less than that to the visceral organs, one might expect the muscle to retain the strontium for a comparatively longer period. The fact that the dark muscle of tuna retained much less strontium than did the light muscle (Table 1), and the fact that the dark muscle is better supplied with blood than is the light muscle, suggest that the degree of vascularization is of some importance. One might therefore reasonably expect the cartilaginous tissues, such as the gill rakers and gill rays, to retain the strontium for longer periods than does the bone which is better supplied with capillaries.

Although the amount of strontium in tuna muscles and visceral organs is of the same order of magnitude (Goldberg, personal communication), some specific binding of strontium may occur with muscle protein which does not occur with the proteins of the visceral organs. Further, the very long biological half-life of strontium in the muscle suggests that fish muscle may not be in such a "dynamic state" as one ordinarily assumes according to the researches of Schoenheimer and his associates (Schoenheimer, 1942). Moreover, there is evidence that mammalian muscle protein has a very much slower turnover rate than has the proteins of the visceral organs (Tarver and Schmidt, 1942). Therefore, the slow turnover of strontium in the fish muscle may be only a reflection of the slow turnover of muscle in general.

#### E. *The uptake and accumulation of $\text{Sr}^{89}$ in solution by *Tilapia**

Because the pattern of distribution of  $\text{Sr}^{89}$  in the tissues and organs of several species of fish appears to be similar, both when the isotope was given orally and by injection, one might extrapolate and conclude that regardless of the mode of entry, the internal distribution of  $\text{Sr}^{89}$  ultimately would be the same. However, to secure more information on this point, and to ascertain whether or not fish could take up strontium directly from the sea water, a situation which is possible in nature, the experiments described below were carried out.

Six *Tilapia* were put into each of four tanks containing 20 liters of filtered sea water and 1744  $\mu\text{c}$  of  $\text{Sr}^{89}$ . The water was aerated during the experiment, but the fish were not fed. The total amount of  $\text{Sr}^{89}$  available during the experiment can be considered constant, since even at 21 days, the total  $\text{Sr}^{89}$  removed by six fish in a tank was less than one per cent of the available dose.

Figure 8 shows the rate of uptake from solution in terms of microcuries of  $\text{Sr}^{89}$ /gram fresh weight of fish. The rate slows down considerably after about a week, but uptake is apparently still continuing. The ordinate on the right indicates that within 21 days, the ratio of internal  $\text{Sr}^{89}$  to external  $\text{Sr}^{89}$  is still less than one.

The internal distribution of the  $\text{Sr}^{89}$  taken up from solution is shown in Figure 9. The  $\text{Sr}^{89}$  found in the skeleton is about 40 per cent as compared with a value close to 60 per cent when the strontium is fed or injected. The amount found in

the integument in all cases is about the same, and the gills and muscles show little variation. The amount found in the visceral organs, however, is markedly different. The individual organs were not ashed and counted separately because of their very small size, so it is not possible to state in what organ or organs the very large percentage of  $\text{Sr}^{89}$  was located.

Since marine fish in general swallow more water than do fresh water fish to maintain proper osmotic balance, it is possible that the principal route of entry of the isotope in solution is by way of the gut. However, our direct feeding experi-

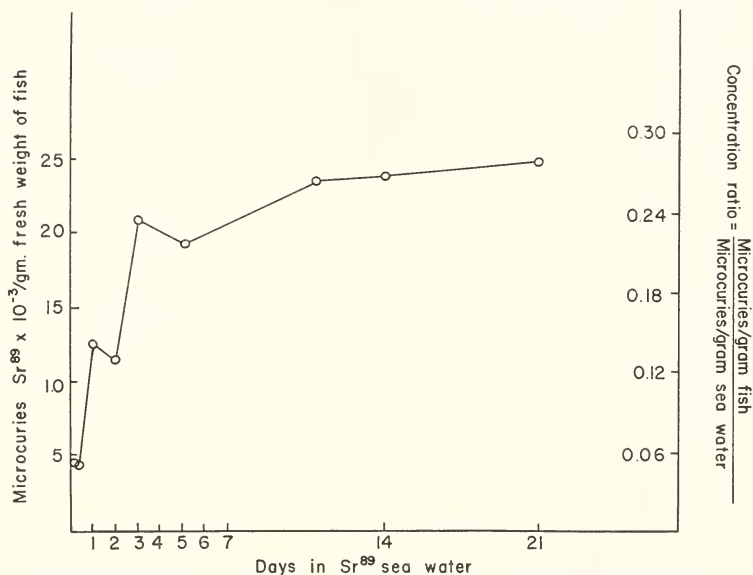


FIGURE 8. The uptake of  $\text{Sr}^{89}$  in solution by *Tilapia mossambica*, expressed as the concentration ratio.

ments with a variety of fishes indicate that strontium is rapidly eliminated from all the visceral organs. How is it, then, that so much strontium remains in the visceral organs when the fish is immersed in the isotope? A probable explanation is that the fish is being fed the isotope continually, in effect, every time it swallows. Since the concentration of  $\text{Sr}^{89}$  was found to be higher in the sea water than in the fish, only a small amount of sea water present in the gut would account for the large percentage of total  $\text{Sr}^{89}$  which was found in the visceral organs. The concentration of  $\text{Sr}^{89}$  in the sea water was  $8.7 \times 10^{-2} \mu\text{c}/\text{ml}$ . Assuming arbitrarily that 50 per cent of the total  $\text{Sr}^{89}$  of the fish was in the visceral organs (Fig. 9), this amount equals about  $12 \times 10^{-3} \mu\text{c}/\text{gram fresh weight of the organs}$ . If only from 0.1–0.2 ml. of sea water was present in the gut, this would account for the radioactivity

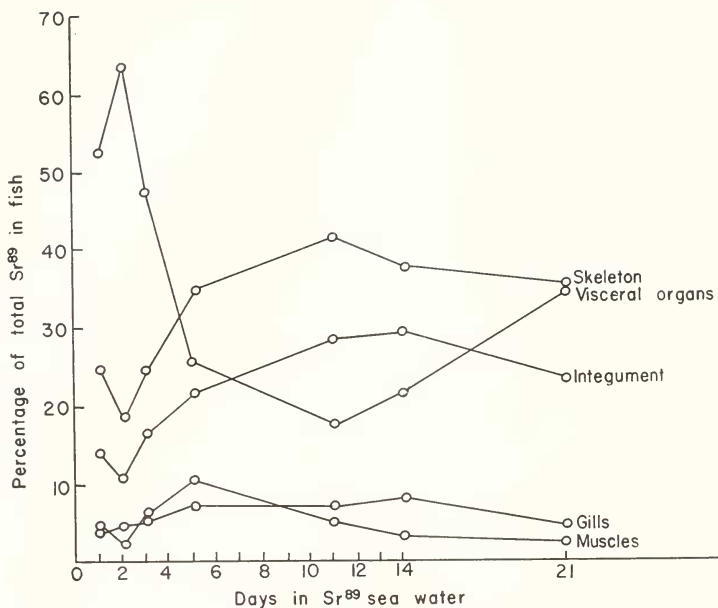


FIGURE 9. The internal distribution of Sr<sup>89</sup> taken up by *Tilapia mossambica* from sea water.

found. What is important, then, is the rank order of radioactivity in the various organs and systems. Excluding the visceral organs for the reasons given above, it is seen that the other organ systems studied fall in the same rank order regardless of the mode of entry of the radiostrontium.

#### SUMMARY

1. The ingestion of Sr<sup>89</sup> by large pelagic fishes results in the excretion of most of the isotope in a few hours. The small percentage remaining after one day persisted for the 27 days of the experiment. The strontium is rapidly eliminated from the visceral organs and tissues, but the structural tissues, including the bones, gills, integument and muscle, maintain their strontium level more or less constant. The turnover of strontium in these latter tissues is therefore slow.

2. Dark muscle, which has a better blood supply than light muscle, retains less Sr<sup>89</sup>. Similarly, bone, which is better supplied with blood than is cartilage, retains less Sr<sup>89</sup> than the gill arches or the cartilaginous eye ossicles.

3. The excretion of Sr<sup>89</sup> by *Tilapia mossambica* is much slower than it is by the pelagic fishes. The percentage of the dose retained is somewhat larger, and most of the radioactivity is found in the structural tissues.

4. About three times as much  $\text{Sr}^{89}$  was found in the muscle of an injected tuna as compared with another fish receiving the isotope orally. The gills of the former fish had only about half the activity found in the latter. From 60-70 per cent of the dose injected into *Tilapia* muscle was retained by these fish for 14 days. The long biological half-life of  $\text{Sr}^{89}$  in fish muscle is contributing evidence for the slow turnover of muscle tissue in comparison with such tissues as liver or kidney.

5. *Tilapia* were able to concentrate  $\text{Sr}^{89}$  directly from the sea water, although the ratio of  $\text{Sr}^{89}$  in the fish to the  $\text{Sr}^{89}$  in an equal weight of sea water was only about 0.3 after three weeks. Except for the visceral organs, the rank order of the retention of radioactivity in the various tissues is skeleton, integument, gills and muscle. This is the same distribution as was observed after oral administration of  $\text{Sr}^{89}$ . Because marine fish swallow water continually, a small amount of water in the gut might account for the relatively large percentage of radioactivity found in the viscera.

#### LITERATURE CITED

- JONES, D. C., AND D. H. COPP, 1951. The metabolism of radioactive strontium in adult, young, and rachitic rats. *Biol. Chem.*, **189**: 509.
- KRUMHOLZ, LOUIS A., 1956. Observations on the fish population of a lake contaminated by radioactive wastes. *Bull. Amer. Mus. Nat. Hist.*, **110**(4): 281-367.
- NATIONAL BUREAU OF STANDARDS HANDBOOK 52, 1953. Maximum permissible amounts of radioisotopes in the human body and maximum permissible concentrations in air and water.
- SCHOENHEIMER, R., 1942. The dynamic state of body constituents. Harvard Univ. Press, Cambridge, Mass.
- TARVER, H., AND C. L. A. SCHMIDT, 1942. Radioactive sulfur studies. *Biol. Chem.*, **146**: 69.
- TESTER, ALBERT L., AND MICHIO TAKATA, 1953. Contribution to the biology of the aholehole, a potential baitfish. *Ind. Res. Advis. Council, Terr. of Hawaii, Grant No. 29, Final Report*.
- TESTER, ALBERT L., AND S. M. TREFZ, 1954. The food of the aholehole, *Kuhlia sandvicensis* (Steindachner), in Hawaiian waters. *Pacific Science*, **8**(1): 3-10.